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(54) Title: METHODS FOR THE PREVENTION AND/OR THE TREATMENT OF NEUROLOGICAL DISORDERS

(57) Abstract: The present invention relates to the use of substrates of an UDP-glucuronosyltransferase (UGT) and salts thereof, for the prevention and/or the treatment of neurological disorders. In preferred embodiment, the present invention relates to the use of substrates of at least an UDP-glucuronosyltransferase (UGT) expressed in brain and salts thereof, for the prevention and/or the treatment of neurological disorders. It further relates to the use of said substrates, and salts thereof, for preventing and/or treating glutamate cytotoxicity, and more specifically of glutamate induced neurological disorders. Additionally, it concerns the use of said substrates, and salts thereof, for making drugs exerting an inhibitory effect on the release of glutamate.

METHODS FOR THE PREVENTION AND /OR THE TREATMENT OF  
NEUROLOGICAL DISORDERS.

The present invention relates to the use of substrates of an UDP-glucuronosyltransferase (UGT) and salts thereof, for the prevention and /or the treatment of neurological disorders. In preferred embodiment, the present invention  
5 relates to the use of substrates of at least an UDP-glucuronosyltransferase (UGT) expressed in brain and salts thereof, for the prevention and /or the treatment of neurological disorders. It further relates to the use of said substrates, and salts thereof, for preventing and/or  
10 treating glutamate cytotoxicity, and more specifically of glutamate induced neurological disorders. Additionally, it concerns the use of said substrates, and salts thereof, for making drugs exerting an inhibitory effect on the release of glutamate.

15 A large number of studies have established that cellular communication using excitatory amino acids can be transformed into a mechanism of cell destruction.

Glutamate, for example, is the main excitatory neurotransmitter in the nervous system, especially brain  
20 and spinal cord, of mammals wherein it is working at a variety of excitatory synapses.

The ubiquitous distribution of glutamate receptors throughout the nervous system proves that glutamate plays

a central role in a wide range of physiological as well as pathological events (Watkins J. C., Collingridge G. L., The NMDA receptor, IRL Oxford, 1989). It is for example strongly suggested that it plays a central role in  
5 functions such as learning, pattern recognition, and memory (Bliss T. V. P. Collingridge G. L., Nature 361, 31-39, 1993).

Normally extracellular levels of glutamate are elevated only in a brief and spatially localized fashion  
10 associated with normal synaptic transmission; however, under pathologic circumstances levels may remain dramatically increased.

Additionally, it has also been known for decades that glutamate is toxic to neurons *in vitro* and *in vivo* and  
15 that the function of glutamate receptors, especially glutamate receptors of the N-methyl-D-aspartate ("NMDA") receptor subtype, is crucial in a number of neuronal damages and injuries (Appel S. H., Trends Neurosci. 16, 3-5, 1993). Many neurological disorders involving epileptic  
20 seizures and chronic or acute degenerative processes, such as for example Alzheimer's, Huntington's, Parkinson's diseases, multiple sclerosis (MS), amyotrophic lateral sclerosis (ALS), spinal muscular atrophy (SMA), retinopathy, stroke, depression and traumatic brain  
25 injury, involve neuronal cell death caused by overstimulation of the glutamate receptors. Similarly, it has been shown that neuronal injury caused by ischemia after occlusion of cerebral arteries could, at least partially, be mediated by excessive activation of glutamate receptors  
30 as in the ischemic brain, extracellular glutamate is elevated rapidly after the onset of ischemia and declines

following reperfusion (Davalos et al., 1997, Stroke, 28, 708-710). Other pathologic circumstances associated with dramatic increase of extracellular glutamate levels are hypoxia or hypoglycaemia. Finally, Stephans and Yamamoto  
5 (1994, Synapse, 17, 203-209) have shown that drug-induced neurotoxicity, for example neurotoxic effects of methamphetamine (METH) on striatal dopaminergic neurons, could actually be mediated by over-stimulation of the glutamate receptors.

10 These excessive activations of glutamate receptors, referred to as "glutamate cytotoxicity", are actually associated with the elevation of extracellular glutamate levels. The mechanisms of the elevation of extracellular glutamate include enhanced efflux of glutamate and/or the  
15 reduction of glutamate uptake by cells. Thus, it would be desirable to provide a means of protecting affected cells, especially neurons, from glutamate-induced cytotoxicity, and more specifically to provide means of regulating glutamate release and/or uptake by glutamate producing  
20 cells.

To this end, it has already been proposed to target the glutamate receptors, mostly the N-methyl-D-aspartate ("NMDA") receptor, present on the targeted cells by inhibiting them by the use of agonist or antagonist  
25 specific molecules. Examples of such molecules are anthranilic acid derivatives (see US 5,789,444), Basilen Blue D-3G (Reactive Blue 2) and Cibacron Blue 3GA and 5-adenylylimidodiphosphate (AMPPNP) (see US 6,326,370), NMDA specific antagonists such as ketamine, gluconate  
30 (Sakaguchi et al., 1999, Neuroscience, 92, 677-684), dextromophan, or 3-(2-carboxypiperazin-4-yl)-propyl-1-

phosphonic acid (Kristensen et al., 1992, Pain, 51:249-253; Eide et al., 1995, Pain, 61,221-228), or the 2-methyl-6-(phenylethynyl)pyridine (MPEP) which is an antagonist of the metabotropic glutamate receptor subtype  
5 5 (mGluR5) (Ossowska et al., 2001, Neuropharmacology,41,413-420).

However, widespread use of these compounds is precluded by their undesirable side effects (e.g. psychotomimetic effects, headache, hallucinations,  
10 dysphoria or disturbances of cognitive and motor functions). Thus, the available treatment methods are not satisfactory in terms of safety or efficiency for their wide implementation.

Therefore, there is still a need in the provision of  
15 improved methods and means for protecting affected cells, and more preferably neurons, from glutamate-induced cytotoxicity.

Similarly, many neurological disorders are associated with elevated levels of neuromediator (or neurotransmitter)  
20 release such as for example acetylcholine (Ach), butyrylcholine (BuCh), gamma-aminobutyrate (GABA), serotonin or the catecholamines, in particular dopamine or ATP.

In mammals, glucuronidation represents a major  
25 metabolic pathway which enhances the detoxification and elimination of many lipophilic drugs, pollutants, xenobiotics and endogenous compounds by converting them in less biologically available, and thus less active, glucuronidated substances exhibiting higher polarity and  
30 water-solubility, thus facilitating their transport to

excretory organs and subsequent excretion in urine or bile (for a review, see Ritter, 2000, Chemico-Biological Interactions, 129, 171-193). The reaction is catalyzed by UDP-glucuronosyltransferases (UGTs). This multigenic  
5 family of enzymes transfer the glucuronic acid moiety of UDP-glucuronic acid to structurally unrelated compounds through hydroxyl (alcoholic, phenolic), carboxyl, sulfuryl, carbonyl and amino (primary, secondary or tertiary) linkages (for review, see Tukey and Strassburg,  
10 2000, Annu. Rev. Pharmacol., Toxicol., 40, 581-616). Initially, glucuronidation has been considered to represent a metabolic pathway performed mainly in the liver. However, multiple studies have further indicated that UGT activity was also present in human intestinal,  
15 kidney, colon tissue and brain where this enzyme is believed to take part actively in the defence of the organism against potentially reactive hydroxylated molecules which can reach the organ (Suleman et al., 1998, Archives of Biochemistry and Biophysics, 358, 1, 63-67 ;  
20 Gradinaru et al., 1999, Neurochemical Research, 24, 995-1000).

The inventors have now shown that the UGT activity in vivo, and more specifically in brain, can be turned away and used for defining a new class of compounds for the  
25 treatment and/or prevention of acute and chronic neuromediator-related diseases or conditions, particularly neurological diseases. The present invention is a pharmacological alternative to previously described methods implementing compounds, such as competitive and  
30 non-competitive glutamate antagonists or agonists, gangliosides and growth factors. In special embodiments,

the present invention provides a new class of compounds which can be used as pharmacological tools for the modulation of neuromediator cellular release and cytotoxicity, preferably neurotoxicity, and which allows  
5 the possible treatment and/or prevention of many neurological disorders involving epileptic seizures and acute and chronic neurodegenerative diseases, as well as neuronal injury caused by ischemia or neuromediator-related diseases or conditions, wherein said disorders  
10 are, at least partially, associated with excessive activation of neuromediator receptors and/or with excessive extracellular neuromediator levels.

First, the invention concerns the use of at least one substrate of an UDP-glucuronosyltransferase (UGT), and  
15 salts thereof, for the preparation of a pharmaceutical composition having an inhibitory effect on the extracellular neuromediator release into an individual treated with said composition. According to one special embodiment, said UDP-glucuronosyltransferase (UGT) is  
20 expressed in brain.

According to specific embodiment, said UDP-glucuronosyltransferase (UGT) is selected among the group consisting of UDP-glucuronosyltransferase 1A1 (UGT1A1, also termed HUG-Br1 or UGT1.1), UDP-  
25 glucuronosyltransferase 1A3 (UGT1A3, also termed 1c), UDP-glucuronosyltransferase 1A4 (UGT1A4, also termed HUG-Br2), UDP-glucuronosyltransferase 1A6 (UGT1A6), UDP-glucuronosyltransferase 1A9 (UGT1A9, also termed HLUG P4), UDP-glucuronosyltransferase 2B4 (UGT2B4, also termed Hlug-  
30 25, Th-1, h-1, or h-20), UDP-glucuronosyltransferase 2B7 (UGT2B7), UDP-glucuronosyltransferase 2B10 (UGT2B10, also

termed h-46), UDP-glucuronosyltransferase 2B15 (UGT2B15, also termed UDPGTh-3,h-3, HLUG-4, or HE 8A), UDP-glucuronosyltransferase 2B17 (UGT2B17) and UDP-glucuronosyltransferase 2B28 (UGT2B28). According to  
5 specific embodiment, said UDP-glucuronosyltransferase (UGT) is expressed in brain and is selected among the group consisting of UDP-glucuronosyltransferase 1A6 (UGT1A6) and UDP-glucuronosyltransferase 2B7 (UGT2B7).

According to the present invention, the term "UDP-glucuronosyltransferase (UGT)" refers to enzymes  
10 catalyzing the glucuronidation reaction. Said reaction utilizes UDP-glucuronic acid as a cosubstrate for the formation of glucuronides. All of these terms are widely used in the litterature; for a review, please refer to  
15 Tukey and Strassburg, 2000, Annu. Rev. Pharmacol. Toxicol., 40, 581-616. The method of choice for analysing the activity of a define UGT is the expression of the corresponding cDNA in a cell line that express minimal levels of the respective protein. More precisely, cells  
20 are transfected by standard transient transfection methods or by stable introduction of the cDNA into the genome following selection of the cells with an appropriate antibiotic. Then the cells are homogenized in a buffer containing Tris-HCl and MgCl<sub>2</sub> and subjected to sonication.  
25 Cell homogenates are then tested for their ability to glucuronidate series of substrates. The activity and selectivity of the UGT for a substrate could be compared with known specific substrates if available (Rommel and Burchell, 1993, Biochem. Pharmacol., 46, 559-566).

30 Currently, 15 UGT cDNAs have been identified in human, eight UGT1A proteins encoded by the UGT1A locus and seven



proteins encoded by UGT2B genes. "UDP-glucuronosyltransferase (UGT) 1A6" refers to one of the UGT proteins encoded by the UGT1A locus. The human UGT1A6 sequence is presented in SEQ ID NO:1 (Harding et al., 5 1988, Proc. Natl. Acad. Sci. 85, 8381-8385 ). UGT1A6 is also called PNP-UGT, phenol p I 6.2, UGT1\*6, UGT1A06 or HLUGP1. "UDP-glucuronosyltransferase (UGT) 2B7" refers to one of the UGT proteins encoded by the UGT2B locus. The human UGT2B7 sequence is presented in SEQ ID NO:2 (Ritter 10 et al., 1990, J. Biol. Chem., 265, 7900-7906. UGT2B7 is also named Th-2, h-2, Hlug-6, UGT2\*7 or Catechol Estrogen UDPGT.

According to the present invention the term "UGT substrate" generally designates both the aglycone 15 substrate (i.e. compound which can be enzymatically converted, or metabolized, by the UGT enzyme as disclosed above) and the glucuronidated substrate (i.e. compound which has been converted by addition of at least one glucuronic acid moiety through active groups).

20 According to the present invention the term "neuromediator", a synonym of "neurotransmitter", is intended to designate chemicals that transmit information across the junction (synapse) that separates one nerve cell (neuron) from another nerve cell or a muscle. 25 Examples of said neuromediators are acetylcholine (Ach), butyrylcholine (BuCh), gamma-aminobutyrate (GABA), serotonin, norepinephrine, epinephrine, endorphins, or the catecholamines, in particular dopamine or adenosine triphosphate. According to one preferred embodiment, said 30 neuromediator is the glutamate.

According to one embodiment, the UGT substrate of the invention is selected among the group consisting of planar and small phenols, polycyclic aromatic hydrocarbons, and compounds which are structurally related. More specifically, said UGT substrate contains at least one moiety selected in the group consisting in hydroxyl (alcoholic, phenolic, etc...), carboxyl, sulfuryl, carbonyl and amino (primary, secondary or tertiary) moieties.

According to a specific embodiment, said substrate is a compound which can be converted by at least the UDP-glucuronosyltransferase 1A6 (UGT1A6) and is selected in the group consisting of 1-Naphthol, 2-Naphthol, 4-nitrophenol, methylsalicylate, ketoprofen, naproxen, 5-OH tryptamine/serotonin, carprofen/rimadyl, acetaminophen/paracetamol, benzidine, 4-methylumbelliferone (4-MU), silymarin (Venketaramanan et al., 2000, Drug Metab Dispos., 28,1270-3 ; silymarin is a mixture of toxifolin, silichristin, silidianin, silybin A et B, isosilybin A et B) [see Figure 1A and 1B].

According to another embodiment, said substrate is a compound which can be converted by at least the UDP-glucuronosyltransferase 2B7 (UGT2B7) and is selected in the group consisting of transretinoic acid, ASA, AZT, benoxaprofen, benzidine, (benzo(a)pyrene mbs), buprenorphine, carprofen, chloramphenicol, clofibric acid, flavenoids quercetin, kaempfenol, cyclosporin, DMXAA, diclofenac, dihydrocodeine DHC, dihydromorphone, mefenamic acid, fenemate NSAID, mycophenolic acid MCPA mofetil, fenoprofen, hydromorphone, ibuprofen, ketoprofen, linoleic acid, lorazepam, losartan, menthol, morphine 3 and morphine 6, nalbufene, nalmeferene,

naltrindole, nalorphine, naloxone, naltrexone, S-naproxen,  
norcodeine, normorphine, oxycodone, oxymorphone,  
pirprofen, propanolol, S-oxazepam, tacrolimus, temazepam,  
tolcapone, tiaprofenic, valproate, zomepirac, 5-OH  
5 tryptamine.

According to a specific embodiment, said substrate is  
selected in the group consisting of ketoprofen, naproxen,  
5-OH tryptamine/serotonin, carprofen/rimadyl and  
benzidine.

10 In special embodiments, the UGT substrates of the  
invention are further substituted with, one to four,  
identical or different, heteroatoms and/or hetero groups.  
Examples of said heteroatoms and/or hetero groups are O,  
H, alkyl or aryl groups  $C_nH_{n+1}$ , with  $n = 1$  to 5,  $OCH_3$ , N,  
15 halogens (fluorine, chlorine, bromine, or iodine atom), S  
or any labeling element allowing to visualize said  
substrates. These substituting atoms or groups, and their  
uses, are widely known in the art.

The addition salts of the substrates of the invention  
20 comprise conventional salt formed from inorganic or  
organic acids or bases, such as hydrochloric, hydrobromic,  
sulfuric, phosphoric, nitric, perchloric, fumaric, acetic,  
sodium, lithium, potassium, magnesium, aluminium, calcium,  
zinc, ethylenediamine; formic, benzoic, maleic, tartaric,  
25 citric, oxalic, aspartic acid, and alkane-sulfonic acids  
is even mentioned.

The UGT substrates used according to the invention  
are commercially available in many commercial catalogues  
for example in Merck, "Produits chimiques et réactifs"  
30 2002 and Acros Organics "Fine Chemicals" 2002-2003 :

	1-Naphtol :	822289 (Merck)
	2-Naphtol :	822290 (Merck)
	4-Nitrophenol :	820896 (Merck)
5	5OH Tryptamine/serotonin	21502-1000 (Acros)
	4-methylumbelliferone	40549-0010 (Acros)
	All trans retinoic acid	20734-1000 (Acros)
	Chloramphenicol	22792-0250 (Acros)
	Clofibric acid	18909-0500 (Acros)

10 Further suppliers are :

Naproxen : Apranax (FR, Roche) or Naprosyn (US, Roche)  
Carprofen : Rimadyl (Pfizer)  
Ketoprofen : Toprec (FR, Aventis) or Orudis (US, Wyeth).

The newly identified inhibitory properties of these  
15 UGT substrates make them particularly suitable for  
treating and/or preventing diseases, conditions and  
attacks related to deleterious effects of neurotransmitter  
released, and preferably neurological ones. In one special  
embodiment, the UGT substrates of the invention are  
20 suitable for treating and/or preventing diseases,  
conditions and attacks related to deleterious effects of  
glutamate released, and preferably neurological ones.

According to one special embodiment, said diseases,  
conditions and attacks are related to deleterious effects  
25 of neurotransmitter released in excess, and more  
particularly to deleterious effects of glutamate released  
in excess.

Thus the present invention further relates to a method  
for treating and/or preventing neuromediator-evoked  
30 cytotoxicity in a patient in need thereof comprising

administering to said patient a composition containing a therapeutically effective amount of at least one substrate of an UDP-glucuronosyltransferase (UGT) as defined above and a pharmaceutically acceptable carrier. In special  
5 embodiment, said UGT substrate is a compound which can be converted by at least one UDP-glucuronosyltransferase (UGT), and preferably by at least one UDP-glucuronosyltransferase (UGT) expressed in brain.

Preferably, the present invention relates to a method  
10 for treating and/or preventing glutamate-evoked cytotoxicity in a patient in need thereof comprising administering to said patient a composition containing a therapeutically effective amount of at least one substrate of an UDP-glucuronosyltransferase (UGT) as defined above  
15 and a pharmaceutically acceptable carrier. In special embodiment, said UGT substrate is a compound which can be converted by at least one UDP-glucuronosyltransferase (UGT), and preferably by at least one UDP-glucuronosyltransferase (UGT) expressed in brain.

20 According to another embodiment, said UGT substrate is selected among the group consisting of planar and small phenols, polycyclic aromatic hydrocarbons, and compounds which are structurally related. More specifically, said UGT substrate contains at least one moiety selected in the  
25 group consisting in hydroxyl (alcoholic, phenolic, etc...), carboxyl, sulfuryl, carbonyl and amino (primary, secondary or tertiary) moieties.

According to another embodiment, said substrate is a compound which can be converted by the UDP-  
30 glucuronosyltransferase 1A6 (UGT1A6) and is selected in

the group consisting of 1-Naphthol, 2-Naphthol , 4-nitrophenol , methylsalicylate, ketoprofen , naproxen, 5-OH tryptamine/serotonin, carprofen/rimadyl , acetaminophen/paracetamol , benzidine, 4-methylumbelliferone (4-MU), silymarin (Venketaramanan et al., 2000, Drug Metab Dispos., 28,1270-3 ; Silymarin is a mixture of toxifolin, silichristin, silidianin, silybin A et B, isosilybin A et B) [see Figure 1].

10 According to another embodiment, said substrate is a compound which can be converted by the UDP-glucuronosyltransferase 2B7 (UGT2B7) and is selected in the group consisting of transretinoic acid, ASA , AZT , benoxaprofen, benzidine, (benzo(a)pyrene mbs),  
15 buprenorphine, carprofen, chloramphenicol, clofibric acid , flavenoids quercetin, kaempferol, cyclosporin, DMXAA, diclofenac, dihydrocodeine DHC, dihydromorphone, mefenamic acid, fenemate NSAID, mycophenolic acid MCPA mofetil, fenoprofen, hydromorphone, ibuprofen, ketoprofen,  
20 linoleic acid , lorazepam, losartan, menthol, morphine 3 and morphine 6, nalbufene, nalmefene, naltrindole, nalorphine, naloxone, naltrexone, S-naproxen, norcodeine, normorphone, oxycodone, oxymorphone, pirprofen, propanolol, S-oxazepam, tacrolimus, temazepam, tolcapone,  
25 tiaprofenic, valproate, zomepirac, 5-OH tryptamine.

According to a special embodiment, said substrate is selected in the group consisting of ketoprofen, naproxen, 5-OH tryptamine/serotonin, carprofen/rimadyl and benzidine.

30 The term "neuromediator-evoked cytotoxicity" or neurotransmitter-evoked cytotoxicity" within the present

invention is intended to designate cell toxicity associated with excessive activations of the concerned neuromediator receptors. These terms are well known by the one skilled in the art. More specifically, the "glutamate-evoked cytotoxicity" concerns all affected cells expressing glutamate receptors. According to preferred embodiments, these cells are susceptible to be affected by neuromediators are nervous cells (i.e. neuro-cells), preferably neurons. These affected nervous cells are, for example, present in brain, spinal cord, retina, at the neuro-muscular junction, etc ... "Cytotoxicity" means that the cell functions and/or properties are affected, leading to cell malfunctioning, and finally to cell death.

In a particularly preferred embodiment, the method of the invention is intended for treating and/or preventing neuromediator-evoked neurotoxicity, and even more preferably for treating and/or preventing neurodegeneration (i.e. degeneration of nervous cells).

In another particularly preferred embodiment, the method of the invention is intended for treating and/or preventing glutamate-evoked neurotoxicity, and even more preferably for treating and/or preventing neurodegeneration (i.e. degeneration of nervous cells).

The present invention further relates to a method for modulating the release of at least one neuromediator in a patient comprising administering to said patient a composition containing a therapeutically effective amount of at least one substrate of an UDP-glucuronosyltransferase (UGT) and a pharmaceutically acceptable carrier, wherein said substrate is as detailed above. In special embodiment, said UGT substrate is a

compound which can be converted by at least one UDP-glucuronosyltransferase (UGT), and preferably by at least one UDP-glucuronosyltransferase (UGT) expressed in brain.

The present invention further relates to a method for  
5 modulating the release of glutamate in a patient comprising administering to said patient a composition containing a therapeutically effective amount of at least one substrate of an UDP-glucuronosyltransferase (UGT) and a pharmaceutically acceptable carrier, wherein said  
10 substrate is as detailed above. In special embodiment, said UGT substrate is a compound which can be converted by at least one UDP-glucuronosyltransferase (UGT), and preferably by at least one UDP-glucuronosyltransferase (UGT) expressed in brain.

15 "Modulating the release of neuromediator" means that the levels of released neuromediator in non treated patient is different from the one observed after his treatment with the substrates of the invention. According to one embodiment, treatment of the patient with the  
20 substrates of the invention leads to a negative modulation, preferably to the inhibition, of the neuromediator release by the producing cells, and thus to a decreased neuromediator level in the treated patient compared to the same neuromediator level observed before  
25 said treatment.

The present invention further relates to a method for treating and/or preventing disease and/or condition associated with the excessive release of at least one neuromediator in a patient comprising administration to  
30 said patient of a composition containing a therapeutically effective amount of at least one substrate of an UDP-



glucuronosyltransferase (UGT) and a pharmaceutically acceptable carrier, wherein said substrate is as detailed above. In special embodiment, said UGT substrate is a compound which can be converted by at least one UDP-glucuronosyltransferase (UGT), and preferably by at least one UDP-glucuronosyltransferase (UGT) expressed in brain.

The present invention further relates to a method for treating and/or preventing disease and/or condition associated with the excessive release of glutamate in a patient comprising administration to said patient of a composition containing a therapeutically effective amount of at least one substrate of an UDP-glucuronosyltransferase (UGT) and a pharmaceutically acceptable carrier, wherein said substrate is as detailed above. In special embodiment, said UGT substrate is a compound which can be converted by at least one UDP-glucuronosyltransferase (UGT), and preferably by at least one UDP-glucuronosyltransferase (UGT) expressed in brain.

According to the invention, the terms "treating and/or preventing" refer to a process that is intended to produce a beneficial change in the condition of a mammal, e.g., a human, often referred to as a patient. A beneficial change can, for example, include one or more of: restoration of function, reduction of symptoms, limitation or retardation of progression of a disease, disorder, or condition or prevention, limitation or retardation of deterioration of a patient's condition, disease or disorder. Such therapy can involve, for example, nutritional modifications, administration of radiation, administration of a drug, behavioural modifications, and combinations of these, among others.

According to the invention, "disease and/or condition associated with the excessive release of at least one neuromediator" is intended to designate large number of acute and chronic neuromediator-related diseases or conditions, particularly neurological diseases. It designates more specifically epileptic seizures and acute and chronic neurodegenerative diseases, as well as neuronal injury caused by ischemia or neuromediator-related diseases or conditions, wherein said disorders are, at least partially, associated with excessive activation of neuromediator receptors and/or with excessive extracellular neuromediator levels. Examples are involving chronic or acute degenerative disorders, such as for example Alzheimer's, Huntington's, Parkinson's diseases, multiple sclerosis (MS), amyotrophic lateral sclerosis (ALS), spinal muscular atrophy (SMA), retinopathy, stroke, clinical depression and traumatic brain injury, involve neuronal cell death caused by overstimulation of the neuromediator receptors, and more specially of the glutamate receptors. Similarly, neuronal injury caused by ischemia or drug-induced neurotoxicity, for example neurotoxic effects of methamphetamine (METH) on striatal dopaminergic neurons, are indications of the methods according to the present invention. Other indications are the neuromediator-related conditions such as for example pain, hormonal balance, blood pressure, thermoregulation, respiration, learning, pattern recognition or memory, or any disorder subsequent to hypoxia or hypoglycaemia, especially when these indications are glutamate-related conditions.

For example, the treatment of epilepsy, clinical

depression, amyotrophic lateral sclerosis, spinal muscular atrophy (SMA), Huntington's disease, deleterious effect due to excesses of glutamate released as a result of cerebral accidents of traumatic or other vascular origin  
5 will be mentioned.

In the above disclosed embodiments of the invention, most of the compositions and methods were related to the aglycone form of said substrates. Alternatively, the invention further relates to the use of at least one  
10 glucuronidated substrate of an UDP-glucuronosyltransferase (UGT) and salts thereof, for the preparation of a pharmaceutical composition having an inhibitory effect on the extracellular neuromediator release into an individual treated with said composition. According to said  
15 embodiment, the substrate of an UDP-glucuronosyltransferase (UGT) is understood as being glucuronidated before its administration to the patient in need thereof. Said glucuronidation can be obtained by modifying the aglycone substrate either by chemical or  
20 enzymatic way. Chemical glucuronidation can be obtained by using standard chemical method well known by those in the art consisting for example in adding a glucuronide group to the aglycone substrate intended to be used (Lacy and Sainsbury, 1995, Tetrahedron lett., 36,3949-50).  
25 Alternatively, the glucuronidated substrate can be obtained by enzymatic glucuronidation using at least one UDP-glucuronosyltransferase (UGT) able to glucuronidate said aglycone substrate. In preferred embodiment said UDP-glucuronosyltransferase (UGT) is naturally expressed in  
30 brain, e.g. UDP-glucuronosyltransferase (UGT) 1A6 or UDP-glucuronosyltransferase (UGT) 2B7.

According to one embodiment, said glucuronidated UGT substrate is selected among the group consisting of planar and small glucuronidated phenols, polycyclic aromatic glucuronidated hydrocarbons, and compounds which are structurally related. More specifically, said glucuronidated substrate contains at least one moiety selected in the group consisting in hydroxyl (alcoholic, phenolic, etc...), carboxyl, sulfonyl, carbonyl and amino (primary, secondary or tertiary) moieties linked to glucuronic acid moiety.

According to a specific embodiment, said glucuronidated substrate is selected in the group consisting of 1-Naphthol, 2-Naphthol, 4-nitrophenol, methylsalicylate, ketoprofen, naproxen, 5-OH tryptamine/serotonin, carprofen/rimadyl, acetaminophen/paracetamol, benzidine, 4-methylumbelliferone (4-MU), silymarin (Venketaramanan et al., 2000, Drug Metab Dispos., 28,1270-3 ; Silymarin is a mixture of toxifolin, silichristin, silidianin, silybin A et B, isosilybin A et B) and is further glucuronidated.

According to another embodiment, said glucuronidated substrate is selected in the group consisting of transretinoic acid, ASA, AZT, benoxaprofen, benzidine, (benzo(a)pyrene mbs), buprenorphine, carprofen, chloramphenicol, clofibric acid, flavonoids quercetin, kaempferol, cyclosporin, DMXAA, diclofenac, dihydrocodeine DHC, dihydromorphone, mefenamic acid, fenamate NSAID, mycophenolic acid MCPA mofetil, fenoprofen, hydromorphone, ibuprofen, ketoprofen, linoleic acid, lorazepam, losartan, menthol, morphine 3 and morphine 6, nalbuphene, nalmefene, naltrindole, nalorphine, naloxone, naltrexone, S-naproxen,

norcodeine, normorphone, oxycodone, oxymorphone, pirprofen, propranolol, S-oxazepam, tacrolimus, temazepam, tolcapone, tiaprofenic, valproate, zomepirac, 5-OH tryptamine and is further glucuronidated.

5       The addition salts of the glucuronidated substrates of the invention comprise conventional salt formed from inorganic or organic acids or bases, such as hydrochloric, hydrobromic, sulfuric, phosphoric, nitric, perchloric, fumaric, acetic, sodium, lithium, potassium, magnesium,  
10   aluminium, calcium, zinc, ethylenediamine ; formic, benzoic, maleic, tartaric, citric, oxalic, aspartic acid, and alkane-sulfonic acids is even mentioned.

      The present invention further relates to a method for treating and/or preventing neuromediator-evoked  
15   cytotoxicity in a patient in need thereof comprising administering to said patient a composition containing a therapeutically effective amount of at least one glucuronidated substrate of an UDP-glucuronosyltransferase (UGT) as defined above and a pharmaceutically acceptable  
20   carrier.

      The present invention further relates to a method for treating and/or preventing glutamate-evoked cytotoxicity in a patient in need thereof comprising administering to said patient a composition containing a therapeutically  
25   effective amount of at least one glucuronidated substrate of an UDP-glucuronosyltransferase (UGT) as defined above and a pharmaceutically acceptable carrier.

      In a particularly preferred embodiment, said "neuromediator-evoked cytotoxicity" is a neuromediator-

evoked neurotoxicity, and even more preferably a neurodegeneration (i.e. degeneration of nervous cells).

In another particularly preferred embodiment, said "glutamate-evoked cytotoxicity" is a glutamate-evoked neurotoxicity, and even more preferably a neurodegeneration (i.e. degeneration of nervous cells).

The present invention further relates to a method for modulating the release of at least one neuromediator in a patient comprising administering to said patient a composition containing a therapeutically effective amount of at least one glucuronidated substrate of an UDP-glucuronosyltransferase (UGT) and a pharmaceutically acceptable carrier, wherein said substrate is as detailed above.

The present invention further relates to a method for modulating the release of glutamate in a patient comprising administering to said patient a composition containing a therapeutically effective amount of at least one glucuronidated substrate of an UDP-glucuronosyltransferase (UGT) and a pharmaceutically acceptable carrier, wherein said substrate is as detailed above.

The present invention further relates to a method for treating and/or preventing disease and/or condition associated with the excessive release of at least one neuromediator in a patient comprising administration to said patient of a composition containing a therapeutically effective amount of at least one glucuronidated substrate of an UDP-glucuronosyltransferase (UGT) and a

pharmaceutically acceptable carrier, wherein said substrate is as detailed above.

The present invention further relates to a method for treating and/or preventing disease and/or condition associated with the excessive release of glutamate in a patient comprising administration to said patient of a composition containing a therapeutically effective amount of at least one glucuronidated substrate of an UDP-glucuronosyltransferase (UGT) and a pharmaceutically acceptable carrier, wherein said substrate is as detailed above.

The UGT substrates (including aglycone or glucuronidated form) described in the present invention are administered as a composition containing at least one active compound and a pharmaceutically acceptable carrier. In preparing such a composition, any conventional pharmaceutically acceptable carrier can be utilized. The carrier material can be an organic or inorganic inert carrier material suitable for the selected route of administration. Suitable carriers include water, gelatin, gum arabic, lactose, starch, magnesium stearate, talc, vegetable oils, polyalkylene-glycols, petroleum jelly and the like. Furthermore, the pharmaceutical composition may contain other pharmaceutically active agents. Additional additives such as flavoring agents, preservatives, stabilizers, emulsifying agents, salts for varying the osmotic pressure, buffers and the like may be added in accordance with accepted practices of pharmaceutical compounding. Any conventional form such as tablets, capsules, pills, powders, granules, and the like may be used. Advantageously, they are in the form of tablets,

sugar coated tablets, hard gelatin capsules, capsules, granules, for oral administration, or solutions or suspensions for administration via an injectable channel.

The methods of the invention may be carried out by  
5 administering the composition containing substrates (including aglycone or glucuronidated form) of the invention by any route whereby drugs are conventionally administered. Such routes include systemic and local routes. Examples are intravenous, intramuscular,  
10 subcutaneous, intracranial, intraventricular, inhalation (Gradinaru et al., 1999 supra), intraperitoneal, as well as oral routes. Preferably, the method of the invention is carried out via oral or intravenous routes of administration.

15 In accordance with this invention, the substrates described herein (including aglycone or glucuronidated form) are useful in pharmaceutically acceptable oral modes. A preferred oral dosage form comprises tablets, capsules of hard or soft gelatin, methylcellulose or of  
20 another suitable material easily dissolved in the digestive tract. The oral dosages contemplated in accordance with the present invention will vary in accordance with the needs of the individual patient as determined by the prescribing physician. The preferred  
25 oral dosage form is capsules or tablets containing from 50 to 500 mg of a substrate of the invention.

Typical preparations for intravenous administration would be sterile aqueous solutions including water/buffered solutions. Intravenous vehicles include  
30 fluid, nutrient and electrolyte replenishers. Preservatives and other additives may also be present such



as antibiotics and antioxidants. Compositions for bolus i.v. administration may contain up to 10 mg/ml (10,000 mg/liter) of substrate described herein. Compositions for i.v. administration preferably contain from about 50  
5 mg/liter to about 500 mg/liter of at least one substrate described herein.

In carrying out the method of the invention, substrate of the invention (including aglycone or glucuronidated form) is generally given to adults daily, preferably  
10 orally or intravenously, in an amount of from about 5 mg/kg to about 30 mg/kg daily, in single or divided doses, preferably from about 13 mg/kg to about 17 mg/kg daily, with the precise dosage being varied depending upon the needs of the patient. The doses will be adapted according  
15 to the patient and the pathology to be treated and are for example 1 mg-100 mg/day. In general, this therapy is carried out for a period of about three months. Alternatively, the method of the invention may be carried out prophylactically for an indefinite time in those  
20 patients who are have a high risk of suffering an acute neurotoxic event, such as a stroke. For the treatment of an acute neurotoxic event, the patient should be treated in accordance with the method of the invention as soon as possible after the diagnosis of the acute neurotoxic  
25 event, preferably within twelve hours, and most preferably within six hours, of the onset of the neurotoxic event. When the drug is administered orally, it is generally administered at regular intervals.

These drugs are notably administered orally or via an  
30 injectable channel.

According to one special embodiment, the UGT

substrates (including aglycone or glucuronidated form) described in the present invention are modified with specific groups facilitating the passage of said UGT substrate throughout the blood-brain barrier (BBB). One  
5 strategy is lipidization, which involves the addition of lipid-like groups through modification of the hydrophilic part of the substrate structure. The resulting lipid-soluble substrates are transported through the BBB by accessing pores that transiently form within the lipid  
10 bilayer. Another strategy is the addition of hydrophobic groups to enhance BBB transfer by passive diffusion. For examples, addition of methyl groups or acetylation of amine groups increase lipophilicity and brain penetration. A further approach is to modify the substrate so that the  
15 compound has a structure that mimics a nutrient, thus giving it access to one of the specialized carrier-mediated transport systems within the BBB such as amino acid, hexose, vitamin and neuropeptide carriers. An alternative approach is the coupling of the substrates,  
20 via chemical linkers, to small synthetic peptide-vectors that cross the cellular membranes efficiently such as Pegelin or Penetratin (for general revue, see Tamsamani et al., 2000, Pharm. Sci. Technol. Today, 3, 155-162).

According to another embodiment, the substrate or  
25 preparation of the invention (including aglycone or glucuronidated form) is administered to the patient in need in combination with a second compound or formulation aimed at facilitating the transfer throughout the BBB. For examples, the substrate or preparation of the invention is  
30 combined with a hypertonic solution, a biologically active agents such as bradykinin and angiotensin peptides, a

vasoactive substance such as histamine, leukotrienes which have the ability of disrupting BBB transiently. According to the present invention, "combination" or "combined with" means that the substrate or preparation of the invention and the second compound or formulation aimed at facilitating the transfer throughout the BBB can be used simultaneously or consecutively or so as to be staggered over time. Simultaneously refers to a coadministration. In this case, these two essential components can be mixed to form a composition prior to being administered, or can be administered at the same time to the patient in need. It is also possible to administer them consecutively, that is to say one after the other, irrespective of which component is administered first. Finally, it is possible to use a mode of administration which is staggered over time or is intermittent and which stops and restarts at intervals which may or may not be regular. It is pointed out that the routes and sites of administration of the two components can be different. The time interval between the administration and the routes and sites of administration can be defined by the skilled person. It is possible to recommend an interval of from 10 min to 72 h, advantageously of from 30 min to 48 h, preferably of from 1 to 24 h and, very preferably, of from 1 to 6 h.

Pharmacogenomic analysis (see for example Ciotti et al., 1997, Pharmacogenetics, 7, 485-495) have shown that polymorphisms in the UGT genes can result in affected enzyme activity and in lowering or increasing the patient reactivity towards UGT substrates. For example, missense mutations in the UGT1A6 have been identified (A541→G541 and A552→C552) which cause variation in the UGT1A6

activity and therefore variation in the rate of their substrate metabolism (for more detail, see Ciotti et al., 1997, Pharmacogenetics, 7, 485-495). Thus, according to another special embodiment, the treatment and/or prevention methods of the present invention further comprise a preliminary step consisting in establishing if the patient to be treated is presenting a genetic polymorphism resulting in less or improved UGT activity. More specifically, said preliminary step consists in determining by nucleotide sequence analysis of the patient genomic DNA if the UGT1A6 gene of said patient to be treated comprises at least one of the mutation A541→G541 (T181A mutation) or A552→C552 (R184S mutation). Details of said method are disclosed for example in Ciotti et al., 1997, Pharmacogenetics, 7, 485-495 the full content of which is incorporated hereby by reference.

All publications and patent applications cited in this specification are herein incorporated by reference as if each individual publication or patent application were specifically and individually indicated to be incorporated by reference. Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be readily apparent to those of ordinary skill in the light of the teachings of this invention that certain changes and modifications may be made thereto without departing from the spirit or scope of the appended claims.

The invention has been described in an illustrative manner, and it is to be understood that the terminology which has been used is intended to be in the nature of words of description rather than of limitation.

Obviously, many modifications and variations of the present invention are possible in light of the above teachings. It is therefore to be understood that within the scope of the appended claims, the invention may be  
5 practised otherwise than as specifically described. Accordingly, those skilled in the art will recognize, or able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described specifically  
10 herein. Such equivalents are intended to be encompassed in the scope of the following claims.

These and other embodiments are disclosed or are obvious from and encompassed by the description and examples of the present invention. Further literature  
15 concerning any one of the methods, uses and compounds to be employed in accordance with the present invention may be retrieved from public libraries, using for example electronic devices. For example the public database "Medline" may be utilized which is available on Internet,  
20 e.g. under <http://www.ncbi.nlm.nih.gov/PubMed/medline.html>. Further databases and addresses, such as <http://www.ncbi.nlm.nih.gov/>,  
<http://www.infobiogen.fr/>, [http://www.fmi.ch/biology/research\\_tools.html](http://www.fmi.ch/biology/research_tools.html), <http://www.tigr.org/>, are known to the  
25 person skilled in the art and can also be obtained using, e.g., <http://www.lycos.com>. An overview of patent information in biotechnology and a survey of relevant sources of patent information useful for retrospective  
30 searching and for current awareness are given in Berks, TIBTECH 12 (1994), 352-364.

## EXAMPLES

*Primary cortical neuron cultures.* Cortices from foetal Wistar rats (E16) were dissected and maintained in PBS without calcium and magnesium. They were then treated  
5 in PBS containing 0.25% Trypsin for 15 min at 37°C and placed in a complete medium containing Horse Serum for inhibiting trypsin activity. The cortices were then dissociated in complete medium (Neurobasal, 2% Horse Serum, 2mM Glutamine, B27 supplement (1X), 100µg/ml  
10 Gentamicyn, 10µM β Mercaptoethanol). Cells were seeded at 10<sup>5</sup> cells per well in a 96 well plate previously coated with Poly-Ornithine at 9µg/ml. The cultures were maintained at 37°C in a humidified incubator with 5% CO<sub>2</sub>. At day 2, 5µM Cytosine Arabinofuranoside was added to the  
15 cell cultures in order to inhibit glial cell proliferation. At day 7, the primary cultures of neurons were available for further experiments.

*Assessment of neuron cell death.* Neuron cell death was estimated by measuring the release of the cytosolic  
20 enzyme, lactate dehydrogenase (LDH), into the medium of cell cultures. The LDH release quantification was performed using the colorimetric CytoTox96<sup>®</sup> nonradioactive assay (Promega). Briefly, 100µL of cell culture medium were removed and centrifuged for 4 min at 1500 rpm in  
25 order to remove cellular debris. 50µL were then added to 50µL of assay buffer and placed in the dark for 30min at room temperature. The reaction was stopped with 50µL of stop solution and the absorbance at 490nm was measured. The percentage of cell death was calculated by comparison  
30 with the control.

*Assessment of glutamate release.* Glutamate released by neurons in culture was evaluated in aliquots of the supernatants by means of a chemiluminescent enzymatic assay (Israel et al., Neurochem Int. 1993 Jan;22(1):53-8).

#### EXAMPLE 1

##### Neuroprotection assay.

Diazepam (Sigma) and the glucuronide derivative of Diazepam, named Temazepam (Alltech), a substrate of the UDP-glucuronosyltransferase 2B7 (UGT2B7), were used to illustrate the preventing and/or treating effects of UGT2B7 substrates on glutamate-induced neurological disorders.

Diazepam and Temazepam were used to protect primary cultures of neurons, isolated from the rat brain cortex, from neurotoxin-induced cell death.

The neurons were treated *in vitro* with 3-nitropropionic acid (3-NP, Sigma) at 400 $\mu$ M, a toxin which induces epileptic seizures in animal models, in absence or presence of Diazepam and Temazepam at 0.1, 1 and 5 $\mu$ M. After 24 hours, the cultures of cells were analysed for the level of toxin-evoked cell death by means of lactate dehydrogenase release quantification.

The results (Figure 2) show that Diazepam protects neurons from 3-NP at all tested doses but that Temazepam significantly reinforces said cell protection by about 50% at 1 and 5 $\mu$ M. These data indicate that glucuronide modification of Diazepam influences cell survival upon

exposure to a toxic compound.

Similar results were obtained with another neurotoxin, the kainic acid at 100 $\mu$ M (Fisher Bioblock), which mimics glutamate-induced epileptic seizures in  
5 animal models. A better neuroprotection was observed with 1 and 5 $\mu$ M of Temazepam compared with Diazepam at the same concentrations (Figure 3).

## EXAMPLE 2

### 10 Neuroprotection assay.

1-Naphthol (Merck), a substrate of UDP-glucuronosyltransferase 1A6 (UGT1A6), was used to illustrate the preventing and/or treating effects of UGT1A6 substrates on glutamate-induced neurological  
15 disorders.

1-Naphthol was used to protect primary cultures of neurons, isolated from the rat brain cortex, from neurotoxin-induced cell death.

The neurons were treated *in vitro* with 5 $\mu$ M of Ionomycin (Sigma), a calcium ionophore, in presence of  
20 10 $\mu$ M of 1-Naphthol, in absence or in presence of 5 or 10 $\mu$ M of 3-methylcholanthrene (3-MC, Aldrich), an UGT inducer which allows the *in situ* glucuronidation of 1-Naphthol.

The results (Figure 4) show that Naphthol protects  
25 neurons from Ionomycin-evoked cell death and that glucuronidation of 1-Naphthol by 3-MC reinforces, dose dependently, the protective effect of 1-Naphthol.

These data indicate that glucuronide derivatives of UGT1A6 substrates present better neuroprotective effects



than the parent molecules.

Glutamate level determination.

The glutamate released by neurons was followed by an enzymatic assay coupled to a chemiluminescent reaction. In  
5 this experiment, primary cultures of neurons isolated from the rat brain cortex, were treated by 1-Naphthol at 10 $\mu$ M with or without 3-MC at 1 or 5 $\mu$ M. The results (Figure 5) indicate that 1-Naphthol reduces glutamate release and that glucuronidation of 1-Naphthol by 3-MC reinforces,  
10 dose dependently, the decrease of glutamate.

This experiment indicates that the protective effect of glucuronidation on UGT1A6 substrates is associated with an improvement of the decrease of glutamate release.

Figure 1 (A/B) : Illustrates the chemical structures of designed compounds cited throughout the specification.

Figure 2 : Effect of Diazepam and Temazepam on neuron  
5 cell death. Neuron cell death in culture was arbitrarily  
set at 0. The cells were treated with the vehicle (lane 1)  
and with 3-NP at 400 $\mu$ M alone (lane 2) or simultaneously  
with one of the three tested concentrations of Diazepam  
(lanes 3-5) and Temazepam (lanes 6-8). Results indicate  
10 that Temazepam at 1 $\mu$ M (lane 7) and 5 $\mu$ M (lane 8) but not at  
0.1 $\mu$ M (lane 6) improves the neuroprotective effect of  
Diazepam observed at all concentrations (lanes 3-5).

Figure 3 : Effect of Diazepam and Temazepam on neuron  
cell death. Neuron cell death in culture was arbitrarily  
15 set at 0. The cells were treated with the vehicle (lane 1)  
and with kainic acid at 100 $\mu$ M alone (lane 2) or  
simultaneously with one of the three tested concentrations  
of Diazepam (lanes 3-5) and Temazepam (lanes 6-8). Results  
indicate that Temazepam at 1 $\mu$ M (lane 7) and 5 $\mu$ M (lane 8)  
20 but not at 0.1 $\mu$ M (lane 6) improves the neuroprotective  
effect of Diazepam observed at all concentrations (lanes  
3-5).

Figure 4 : Effect of 1-Naphthol and 1-Naphthol  
glucuronide on neuron cell death. Neuron cell death in  
25 culture was arbitrarily set at 0. The cells were treated  
with the vehicle (lane 1) and with Ionomycin at 5 $\mu$ M alone  
(lane 4) or simultaneously with 1-Naphtol at 10 $\mu$ M (lane 7)  
with 3-Methylcholanthrene at 5 $\mu$ M (lane 8) or 10 $\mu$ M (lane  
9). The two tested doses of 3-Methylcholanthrene were  
30 evaluated in absence (lanes 2, 3) or presence (lanes 5, 6)

of Ionomycin and considered as controls. Results indicate that 3-Methylcholanthrene alone has no effect on the natural death of neurons (lanes 2, 3) and that it has no protective effect against the toxicity induced by Ionomycin (lanes 5, 6). Results also show that the protection induced by 1-Naphthol at 10 $\mu$ M (lane 7) is improved by the glucuronidation induced by 3-Methylcholanthrene at 5 $\mu$ M (lane 8) and 10 $\mu$ M (lane 9).

Figure 5 : Effect of 1-Naphthol and 1-Naphthol glucuronide on glutamate released by neurons in culture. Glutamate release was evaluated by means of a chemiluminescent enzymatic assay. The basal levels of glutamate release were quantified in the non-treated cells (lane 1), in cells treated with the vehicle (lane 2) and in cells treated with 3-Methylcholanthrene at the highest dose tested in this experiment, 5 $\mu$ M (lane 3). The effect of 1-Naphthol at 1 $\mu$ M (lane 4) and 10 $\mu$ M (lane 7) was evaluated in presence of 3-Methylcholanthrene at 1 $\mu$ M (lanes 5, 8) and at 5 $\mu$ M (lanes 6, 9). Results indicate that the vehicle and the glucuronidation inducer 3-Methylcholanthrene have no effect on glutamate release (lanes 2, 3) whereas 1-Naphthol at both concentrations reduces the release of glutamate (lanes 4, 7). In addition, the glucuronidation of 1-Naphthol at both doses improves the decrease of glutamate for the two tested doses of 3-Methylcholanthrene (lanes 5, 6, 8, 9). This improvement becomes statistically significant at the highest dose of 3-Methylcholanthrene (lanes 6, 9) whatever the 1-Naphthol dose is.

CLAIMS

1. Use of at least one substrate of an UDP-glucuronosyltransferase (UGT), and salts thereof, for the preparation of a pharmaceutical composition having an inhibitory effect on the extracellular glutamate release into an individual treated with said composition.
2. The use of claim 1, wherein said UDP-glucuronosyltransferase (UGT) is expressed in brain and is selected among the group consisting of UDP-glucuronosyltransferase 1A6 (UGT1A6) and UDP-glucuronosyltransferase 2B7 (UGT2B7).
3. The use of claim 1 or 2, wherein said UGT substrate is selected among the group consisting of planar and small phenols, polycyclic aromatic hydrocarbons, and compounds which are structurally related.
4. The use of claims 1-3, wherein said substrate is a substrate of the UDP-glucuronosyltransferase 1A6 (UGT1A6) and is selected in the group consisting of 1-Naphthol, 2-Naphthol, 4-nitrophenol, methylsalicylate, ketoprofen, naproxen, 5-OH tryptamine/serotonin, carprofen/rimadyl, acetaminophen/paracetamol, benzidine, 4-methylumbelliferone (4-MU), silymarin.
5. The use of claims 1-3, wherein said substrate is a substrate of the UDP-glucuronosyltransferase 2B7 (UGT2B7) and is selected in the group consisting of transretinoic acid, ASA, AZT, benoxaprofen, benzidine, (benzo(a)pyrene mbs), buprenorphine, carprofen, chloramphenicol, clofibric acid, flavenoids quercetin, kaempferol, cyclosporin, DMXAA, diclofenac,

dihydrocodeine DHC, dihydromorphone, mefenamic acid, fenemate NSAID, mycophenolic acid MCPA mofetil, fenoprofen, hydromorphone, ibuprofen, ketoprofen, linoleic acid, lorazepam, losartan, menthol, morphine 3 and  
5 morphine 6, nalbuphene, nalmefene, naltrindole, nalorphine, naloxone, naltrexone, S-naproxen, norcodeine, normorphone, oxycodone, oxymorphone, pirprofen, propanolol, S-oxazepam, tacrolimus, temazepam, tolcapone, tiaprofenic, valproate, zomepirac, 5-OH tryptamine.

10 6. The use of claims 1-5, wherein said pharmaceutical composition is intended for treating and/or preventing glutamate-evoked cytotoxicity in a patient in need.

7. The use of claims 1-5, wherein said pharmaceutical composition is intended for treating and/or preventing  
15 glutamate-evoked neurotoxicity.

8. The use of claims 1-5, wherein said pharmaceutical composition is intended for treating and/or preventing neurodegeneration.

9. The use of claims 1-5, wherein said pharmaceutical  
20 composition is intended for modulating the release of glutamate in a patient.

10. The use of claims 1-5, wherein said pharmaceutical composition is intended for treating and/or preventing disease and/or condition associated with the excessive  
25 release of glutamate in a patient.

11. The use of claim 10, wherein said disease and/or condition associated with the excessive release of glutamate is selected among the group consisting of epileptic seizures, acute and chronic neurodegenerative  
30 diseases, ischemia, Alzheimer's, Huntington's, Parkinson's

diseases, multiple sclerosis (MS), amyotrophic lateral sclerosis (ALS), spinal muscular atrophy (SMA), retinopathy, stroke and traumatic brain injury, drug-induced neurotoxicity, pain, hormonal balance, blood  
5 pressure, thermoregulation, respiration, learning, pattern recognition, memory, and disorders subsequent to hypoxia or hypoglycaemia.

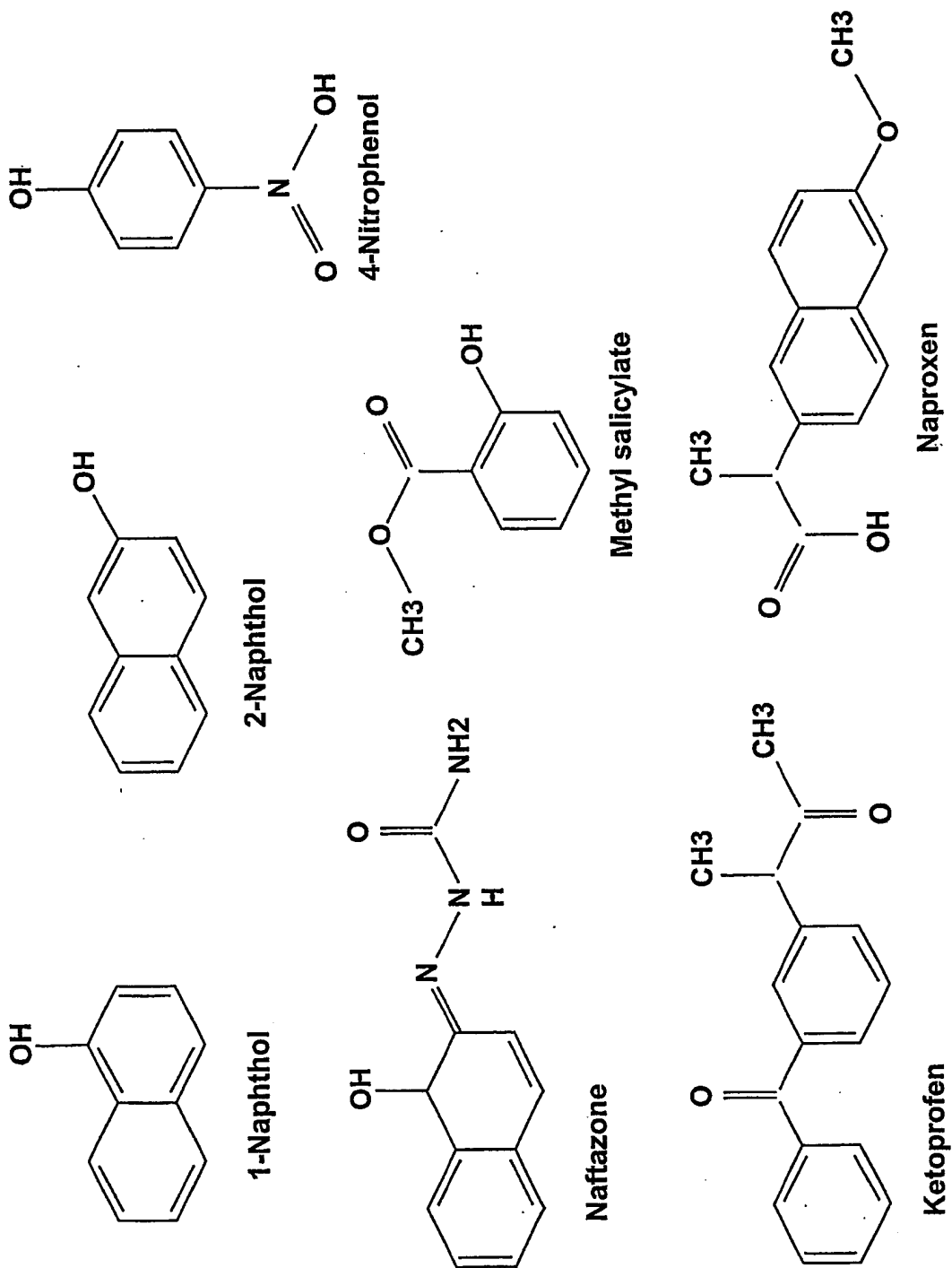
12. Use of at least one glucuronidated substrate of an UDP-glucuronosyltransferase (UGT) and salts thereof, for  
10 the preparation of a pharmaceutical composition having an inhibitory effect on the extracellular glutamate release into an individual treated with said composition.

13. The use of claim 12, wherein said glucuronidated UGT substrate is selected among the group consisting of planar  
15 and small glucuronidated phenols, polycyclic aromatic glucuronidated hydrocarbons, and compounds which are structurally related.

14. The use of claims 12-13, wherein said glucuronidated substrate is selected in the group consisting of 1-Naphthol, 2-Naphthol , 4-nitrophenol , methylsalicylate,  
20 ketoprofen , naproxen, 5-OH tryptamine/serotonin, carprofen/rimadyl , acetaminophen/paracetamol , benzidine, 4-methylumbelliferone (4-MU), silymarin and is further glucuronidated.

25 15. The use of claims 12-13, wherein said glucuronidated substrate is selected in the group consisting of transretinoic acid, ASA , AZT , benoxaprofen , benzidine, (benzo(a)pyrene mbs), buprenorphine, carprofen, chloramphenicol, clofibric acid , flavenoids quercetin,  
30 kaempfenol, cyclosporin, DMXAA, diclofenac, dihydrocodeine

DHC, dihydromorphone, mefenamic acid, fenemate NSAID, mycophenolic acid MCPA mofetil, fenoprofen, hydromorphone, ibuprofen, ketoprofen, linoleic acid , lorazepam, losartan, menthol, morphine 3 and morphine 6, nalbufene, 5 nalmefene, naltrindole, nalorphine, naloxone, naltrexone, S-naproxen, norcodeine, normorphone, oxycodone, oxymorphone, pirprofen, propanolol, S-oxazepam, tacrolimus, temazepam, tolcapone, tiaprofenic, valproate, zomepirac, 5-OH tryptamine and is further glucuronidated.





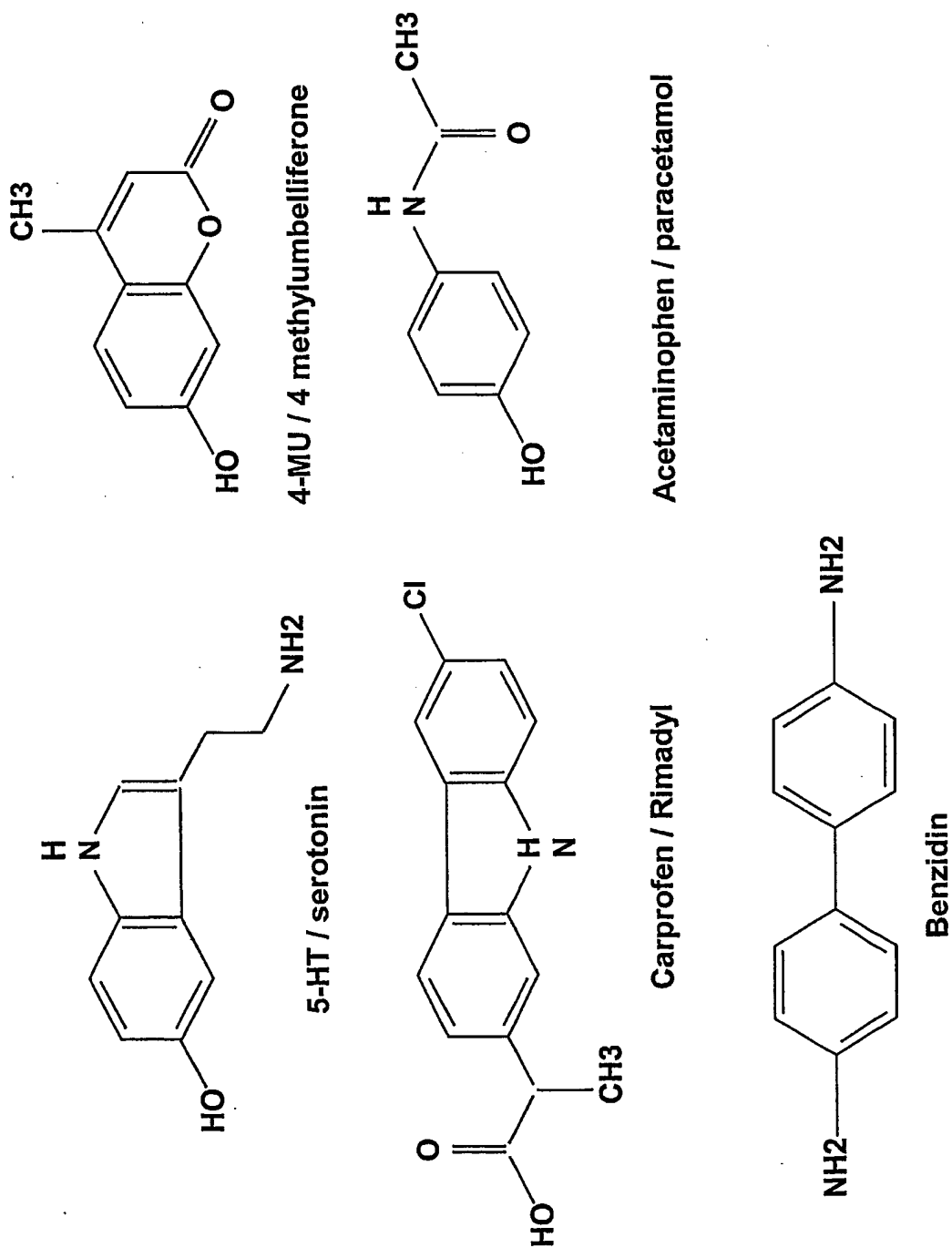


FIGURE 1B

3/6

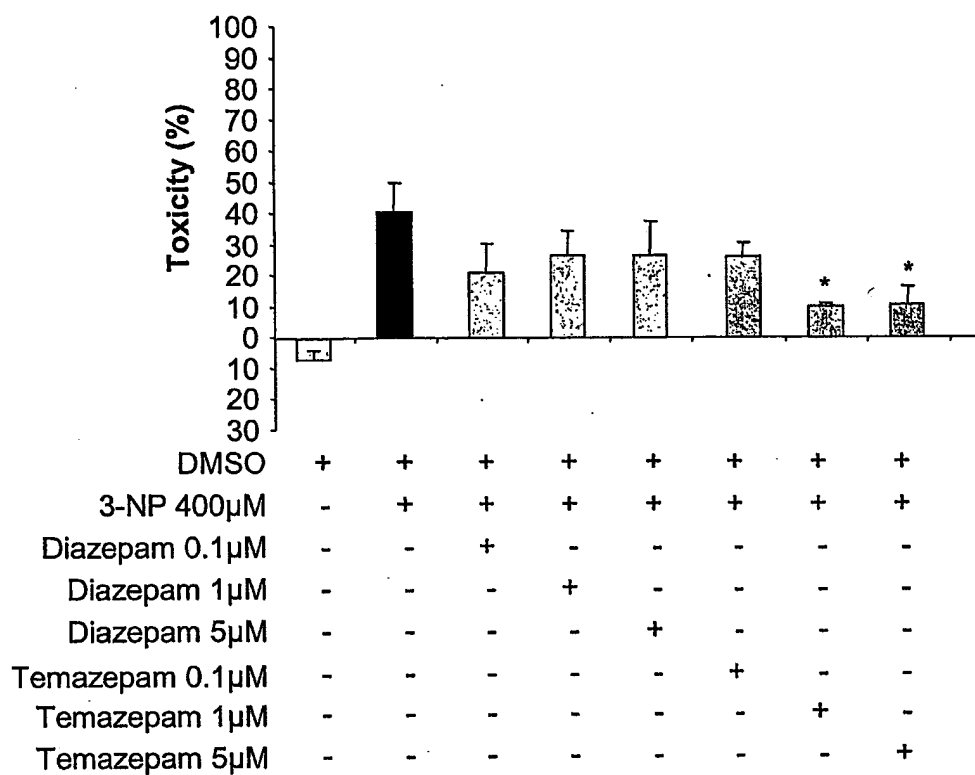


Figure 2

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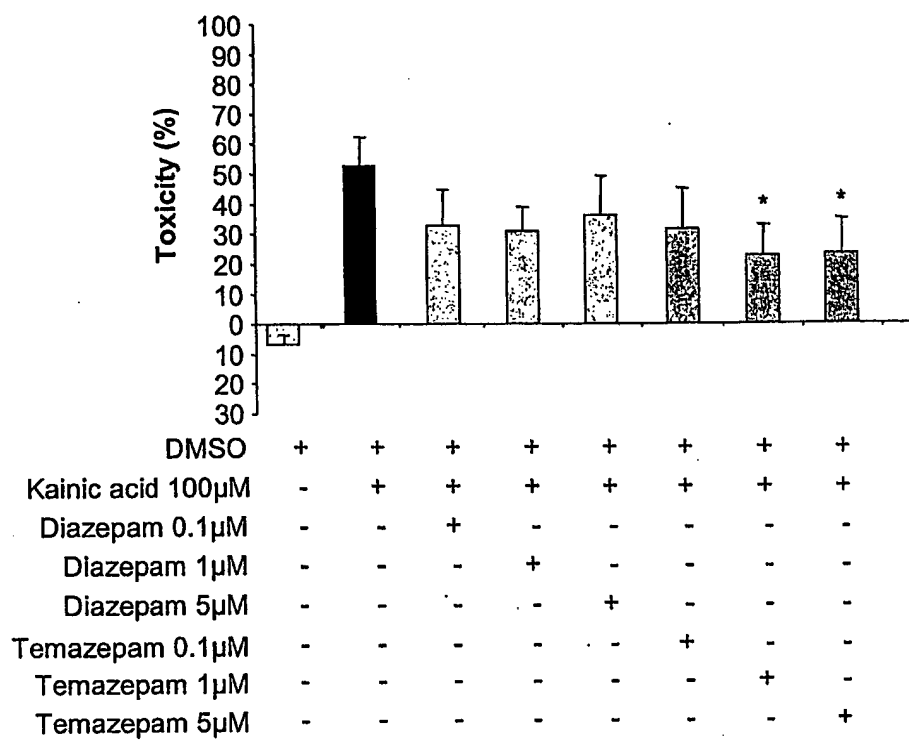


Figure 3

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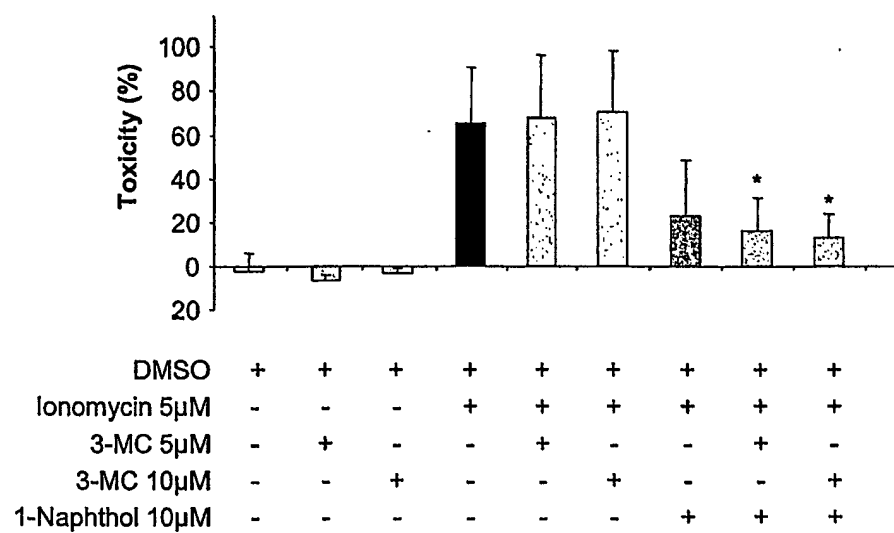


Figure 4

6/6

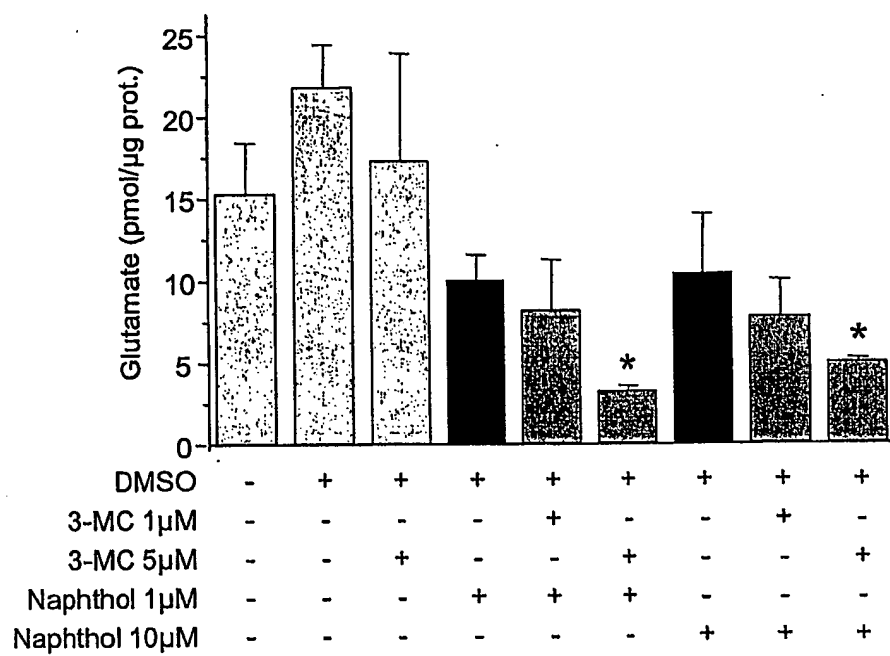


Figure 5

## SEQUENCE LISTING

(1) GENERAL INFORMATION:  
5 (iii) NUMBER OF SEQUENCES: 2  
(2) INFORMATION FOR SEQ ID NO: 1:  
    (i) SEQUENCE CHARACTERISTICS:  
        (A) LENGTH: 531 amino acids  
        (B) TYPE: amino acid  
10 (C) STRANDEDNESS:  
        (D) TOPOLOGY: linear  
    (ii) MOLECULE TYPE: protein  
    (iii) HYPOTHETICAL: NO  
    (iv) ANTI-SENSE: NO  
15 (vi) ORIGINAL SOURCE:  
        (A) ORGANISM: UDP glycosyltransferase 1 family,  
polypeptide A6 [Homo sapiens]  
  
20 (ix) FEATURE:  
    (A) NAME/KEY: UDP glycosyltransferase 1A6  
    (B) LOCATION:  
  
    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1  
25  
1 MACLLRSFQR ISAGVFFLAL WGMVVGDKLL VVPQDGSHWL SMKDIVEVLS  
DRGHEIVVVV  
61 PEVNLLLKEY KYYTRKIYPV PYDQEELKNR YQSGNNHFA ERSFLTAPQT  
EYRNNMIVIG  
30 121 LYFINCQSLL QDRDTLNFFK ESKFDALFTD PALPCGVILA EYGLGPSVYL  
FRGFPCSLEH  
181 TFSRSPDPVS YIPRCYTKFS DHMTFSQRVA NFLVNLLEPY LFYCLFSKYE  
KLASAVLKRD  
241 VDIITLSEVS VWLLRYDFVL EYPRPVMPNM VFIGGINCKK RKDLSQEFEA  
35 YINASGEHGI  
301 VVFSLGSMVS EIPEKKAMAI ADALGKNPQT VLWRYTGTRP SNLANNTILV  
KWLPQNDLLG  
361 HPMTRAFITH AGSHGVYESI CNGVPMVMMP LFGDQMDNAK RMETKGAGVT  
LNVLEMTSED  
40 421 LENALKAVIN DKSYPENIMR LSSLHKDRPV EPLDLAVFWV EFVMRHKGAP  
HLRPAADLT

481 WYQYHSLDVI GFLAVVLTV AFITFKCCPY GYPKCLGKKG RVKKAHKS  
H

- (3) INFORMATION FOR SEQ ID NO: 2:
- 5 (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 529 amino acids
  - (B) TYPE: amino acid
  - (C) STRANDEDNESS:
  - (D) TOPOLOGY: linear
- 10 (ii) MOLECULE TYPE: protein
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO
- (vi) ORIGINAL SOURCE:
- (A) ORGANISM: UDP-glucuronyltransferase, family
- 15 2, beta-7 [Homo sapiens]
- (ix) FEATURE:
- (A) NAME/KEY: UDP glycosyltransferase 2B7
  - (B) LOCATION:
- 20 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:2

1 MSVKWTSVIL LIQLSFCFSS GNCCKVLVWA AEYSHWMNIK TILDELIQRG  
25 HEVTVLASSA  
61 SILFDPNNSS ALKIEIYPTS LTKTELENFI MQQIKRWSDL PKDTFWLYFS  
QVQEIMSIFG  
121 DITRKFCCKDV VSNKKFMKKV QESRFDVIFA DAIFPCSELL AELENIPIFY  
SLSFSPGYTF  
30 181 EKHSGGFIFP PSYVPVVMSE LTDQMTFMER VKNMIYVLYF DFWFEIFDMK  
KWDQFYSEVL  
241 GRPTTLSETM GKADVWLIRN SWNFQFPHPL LPNVDFVGGL HCKPAKPLPK  
EMEDFVQSSG  
301 ENGVVVFSLG SMVSNMTEER ANVIASALAQ IPQKVLWRFD GNKPDTLGLN  
35 TRLYKWIPQN  
361 DLLGHPKTRA FITHGGANGI YEAIYHGIPM VGIPLFADQP DNIAHMKARG  
AAVRVDFNTM  
421 SSTDLLNALK RVINDPSYKE NVMKLSRIQH DQPVKPLDRA VFWIEFVMRH  
KGAKHLRVAA

481 HDLTWFQYHS LDVIGFLLVC VATVIFIVTK CCLFCFWKFA RKAKKGKND



## INTERNATIONAL SEARCH REPORT

PCT/EP 03/05186

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 A61K31/00 A61K31/5513 A61K31/05 A61P25/00

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 A61K A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, PAJ, BIOSIS, EMBASE, CHEM ABS Data, MEDLINE

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	IBRAHIM SAFINAZ S ET AL: "Amelioration of brain ischemia/reperfusion injury by diazepam in rats." EGYPTIAN JOURNAL OF PHARMACEUTICAL SCIENCES, vol. 40, no. 2, 1999, pages 179-196, XP009017816 ISSN: 0301-5068 abstract  --- -/--	1-3,5, 9-11

☒ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

## \* Special categories of cited documents:

- \*A\* document defining the general state of the art which is not considered to be of particular relevance
- \*E\* earlier document but published on or after the international filing date
- \*L\* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- \*O\* document referring to an oral disclosure, use, exhibition or other means
- \*P\* document published prior to the international filing date but later than the priority date claimed

- \*T\* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- \*X\* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- \*Y\* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- \*Z\* document member of the same patent family

Date of the actual completion of the international search

26 September 2003

Date of mailing of the international search report

22/10/2003

Name and mailing address of the ISA

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Pacreu Largo, M

## INTERNATIONAL SEARCH REPORT

PCT/EP 03/05186

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DOWDEN J ET AL: "DIAZEPAM-INDUCED NEUROPROTECTION: DISSOCIATING THE EFFECTS OF HYPOTHERMIA FOLLOWING GLOBAL ISCHEMIA" BRAIN RESEARCH, AMSTERDAM, NL, vol. 829, no. 1/2, 1999, pages 1-6, XP000957597 ISSN: 0006-8993 page 1 -page 2, paragraph 1 page 5, right-hand column, last paragraph	1-3,5-11
X	SWINNEN J: "Epilepsies and medication: Clinical and therapeutic assessment of epilepsies" FARMACEUTISCH TIJDSCHRIFT VOOR BELGIE 1990 BELGIUM, vol. 67, no. 2, 1990, pages 42-54, XP009017817 ISSN: 0771-2367 page 53-54	1-3,5, 10,11
X	REYES AVILA L H: "Treatment of twelve cases of status epilepticus with injectable temazepam" MEDICINA 1972, vol. 52, 1972, pages 468-472, XP001155204 page 471	1-3,5, 10,11
X	DIOS-VIEITEZ M C ET AL: "Pharmacokinetic-pharmacodynamic modeling of the anticonvulsant effect of temazepam "in vivo" in the rat." METHODS AND FINDINGS IN EXPERIMENTAL AND CLINICAL PHARMACOLOGY, vol. 19, no. SUPPL. A, November 1997 (1997-11), page 195 XP009017798 XXI Congress of the Spanish Society of Pharmacology;Barcelona, Spain; November 11-14, 1997 ISSN: 0379-0355 abstract	1-3,5, 10,11
A	WILSON A S ET AL: "Characterisation of the toxic metabolite(s) of naphthalene." TOXICOLOGY. IRELAND 18 DEC 1996, vol. 114, no. 3, 18 December 1996 (1996-12-18), pages 233-242, XP002255889 ISSN: 0300-483X see Discussion abstract	1-4,6-14

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## INTERNATIONAL SEARCH REPORT

PCT/EP 03/05186

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 98 20864 A (MEMO MAURIZIO ;PIZZI MARINA (IT); GRILLI MARIAGRAZIA (IT); SPANO P) 22 May 1998 (1998-05-22) claims	1-11
X	KING C D ET AL: "Expression of UDP-glucuronosyltransferases (UGTs) 2B7 and 1A6 in the human brain and identification of 5-hydroxytryptamine as a substrate." ARCHIVES OF BIOCHEMISTRY AND BIOPHYSICS, vol. 365, no. 1, 1 May 1999 (1999-05-01), pages 156-162, XP002255890 ISSN: 0003-9861 abstract	12-15
X	SHIMOMURA K ET AL: "ANALGESIC EFFECT OF MORPHINE GLUCURONIDES" TOHOKU JOURNAL OF EXPERIMENTAL MEDICINE, vol. 105, no. 1, 1971, pages 45-52, XP009017724 ISSN: 0040-8727 abstract	12-15
A	GREENAMYRE J T: "THE ROLE OF GLUTAMATE IN NEUROTRANSMISSION AND IN NEUROLOGIC DISEASE" ARCHIVES OF NEUROLOGY, vol. 43, no. 10, 1986, pages 1058-1063, XP009017839 ISSN: 0003-9942 the whole document	1-11
A	HUGON J ET AL: "Role of glutamate and excitotoxicity in human neurological disease." REVUE NEUROLOGIQUE (PARIS), vol. 152, no. 4, 1996, pages 239-248, XP009017777 ISSN: 0035-3787 abstract	1-11
A	SULEMAN FUNMILAYO G ET AL: "Identification of the uridine diphosphate glucuronosyltransferase isoform UGT1A6 in rat brain and in primary cultures of neurons and astrocytes." ARCHIVES OF BIOCHEMISTRY AND BIOPHYSICS, vol. 358, no. 1, 1 October 1998 (1998-10-01), pages 63-67, XP002255891 ISSN: 0003-9861 abstract	1-15

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## INTERNATIONAL SEARCH REPORT

PCT/EF 03/05186

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	MARTINASEVIC M K ET AL: "Immunohistochemical localization of UDP-glucuronosyltransferases in rat brain during early development." DRUG METABOLISM AND DISPOSITION: THE BIOLOGICAL FATE OF CHEMICALS. UNITED STATES OCT 1998, vol. 26, no. 10, October 1998 (1998-10), pages 1039-1041, XP002255892 ISSN: 0090-9556 abstract	1-15
A	TUKEY R H ET AL: "HUMAN UDP-GLUCURONOSYLTRANSFERASES: METABOLISM, EXPRESSION, AND DISEASE" ANNUAL REVIEW OF PHARMACOLOGY AND TOXICOLOGY, ANNUAL REVIEW INC., PALO ALTO, CA, US, vol. 40, 2000, pages 581-616, 2PAGES, XP009003008 ISSN: 0362-1642 page 590 -page 595	1-15

# INTERNATIONAL SEARCH REPORT

PCT/EP 03/05186

## Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:
  
2. ☒ Claims Nos.:  
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:  
see FURTHER INFORMATION sheet PCT/ISA/210
  
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
  
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
  
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
  
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

## FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

## Continuation of Box I.2

Present claims 1, 2 and 12 relate to an extremely large number of possible compounds defined by reference to a desirable characteristic or property, namely:

"substrate of an UDP-glucuronosyltransferase (UGT) or glucuronidated substrate of an UGT"

The further definition in claims 3 and 13 ("planar and small phenols, polycyclic aromatic hydrocarbons and compounds structurally related") relates equally to a very large number of possible compounds.

Furthermore, the medical indication is defined by means of a mechanism of action, namely:

"having an inhibitory effect on the extracellular glutamate release"

Such a functional definition is considered to lead to a lack of clarity since it is not possible to list all the diseases that may fall under this definition.

Therefore, present claims relate to an extremely large number of possible compounds to be used for the preparation of a medicament for the treatment of a large number of diseases. In fact, the claims contain so many possible permutations that a lack of clarity and conciseness within the meaning of Article 6 PCT arises to such an extent as to render a meaningful search of the claims impossible.

On top of that, claims 4 and 5 refer to many known compounds which are already well-known for treating some of the specific diseases of claim 11.

Consequently, the search has been based on the compounds of examples 1 and 2 of the application (description, p.30-32) and the diseases specifically claimed in claim 11.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

## INTERNATIONAL SEARCH REPORT

PCT/EP 03/05186

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
WO 9820864	A	22-05-1998	IT WO	MI962356 A1 9820864 A2
				13-05-1998 22-05-1998